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"BIOLOGICAL CONTROL OF METHANE IN COAL MINES, COAL STORAGE SILOS AND SHIP HOLDS PHASE III"

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BY

GEMINI BIOCHEMICAL RESEARCH LTD.

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BIOLOGICAL CONTROL OF METHANE IN COAL MINES COAL STORAGE SILOS AND SHIP HOLDS

PHASE III

A REPORT BY

GEMINI BIOCHEMICAL RESEARCH LTD.

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SUMMARY

The small scale experiments used in the previous study were successfully scaled up to allow treatment of 10 Kg quantities of coal. The rates of methane oxidation in the larger silos were as high as those observed in the small tests. No differences in gas composition were noted if gas samples were withdrawn from either the airspace above the coal or from within the coal pile. This showed that diffusion of methane or oxygen was not limiting the rate of methane oxidation.

The pH activity profile of the strain of organisms used was broadened from that used in the previous study. Significant activity was found at lower pH values, particularily in the range 5.0-6.5. The organisms were most active in the range 6.0-8.0.

The strains of organisms isolated in the previous study were more fully characterized, particularily their temperature and pH profiles. Optimum temperature for bacterial activity was shown to be 33°C. Activity dropped rapidly when the temperature was raised above the optimum but only dropped slowly when it was lowered. The organisms were inactive at extremes of temperature, e.g. -20 and 40°C but recovered when the temperature was adjusted to more favourable values.

Methods were adopted to allow the organisms to be used with high sulphur Cape Breton coals. Specifically, three methods were found to allow the organisms to be used with the Cape Breton coals. Firstly, the pH profile of the organisms was adapted to lower values. Secondly, methods were found to immobilize the organisms so that they did not come in contact with the coal. Thirdly, if the inorganic sulphur was removed the resulting coal did not inactivate the organisms.

ABREGE

Les expériences à petite échelle qui ont servi aux études préalables ont été effectuées sur de quantités de charbon plus importantes , ce qui a facilité le traitement de charbon en quantités de 10 kilos à la fois.

Les taux d'oxydation de méthane dans les silos plus importantes étaient aussi élevés que ceux qu'on avait notés lors des essais plus modestes. On n'a pas constaté de différence dans la composition du gaz lorsqu'on a relevé des échantillons de gaz depuis l'espace d'air au-dessus du charbon ou depuis l'intérieur de l'entassement du charbon.Ceci a donc démontré que le taux d'oxydation de méthane n'a pas été restreint par la diffusion de méthane ou d'oxygène.

Le profil d'activité du pH dans la souche d'organismes utilisée a été agrandi par rapport à celui dont on se servait dans l'étude précédente. On a constaté une activité notable à des indices de pH plus bas, surtout à l'échelle de 5.0 - 6.5. Les organismes se sont montrés actifs surtout à l'échelle de 6.0 - 8.0.

Les souches des organismes isolés lors de l'étude précédente ont été décrites de manière plus ample, surtout quant aux profils de température et de pH. On a démontré que la température optimale qui favorise l'activité bactérienne est de 33 degrés Celsius. Une diminution rapide dans cette activité s'est produite lorsqu'on a élevé la température au-dessus de la température optimale; par contre, cette diminution s'est effectuée lentement lorsqu'on a baissé la température. Les organismes sont restés inactifs à des températures extrêmes, par exemple, à -20 degrés C. et à 40 degrés C.- mais ils sont redevenus actifs lorsque les températures ont été ajustées à des valeurs plus favorables.

On a utilisé des méthodes pour permettre l'emploi des organismes dans le charbon à haute teneur en soufre du Cap Breton. Plus précisément, on a trouvé trois méthodes pour faciliter l'emploi des organismes conjointement avec les charbons du Cap Breton. D'abord, le profil pH des organismes a été adapté à des indices plus bas. Ensuite, on a inventé des méthodes pour

immobiliser des organismes, les empêchant ainsi de rentrer en contact avec le charbon. En troisième lieu, si l'on enlevait le soufre minéral, les organismes restaient inactifs envers le charbon.

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INTRODUCTION

For the past three years, Gemini Biochemical Research Ltd. has been conducting a research project on the biological control of methane in coal mines and coal storage vessels. This project is jointly supported by CANMET and Transport Canada (Ship Safety Division). The results of Phase I included a literature search on the properties of methane oxidizing bacteria to identify if conditions in coal mines and coal storage vessels would be suitable environments for these organisms. In addition, a review of the latest research from Russia, Eastern Europe and India was included.

The literature review showed that methane oxidizing bacteria had physiological properties which were suitable for their use in coal mining environments. In particular, their pH profiles, temperature optima and nutritional requirements were found to be appropriate.

The survey of the recent field tests in various countries showed that most of the tests yielded positive results when used under actual mining conditions but that there was a paucity of information on the microbiological characteristics of the organisms used and that not enough data were presented to critically assess the validity of the results.

Based on the findings presented in the report from Phase I (1), Phase II was initiated and the results of this phase of the project are summarized below (2).

Water and coal samples were obtained from five mines in Western Canada and two from Cape Breton. Chemical analysis of the water samples and coal leachates from the Western Canadian mines showed that conditions in these mines were appropriate for the growth of methane oxidizing bacteria. In particular, the pH of the mine waters was in the correct

range (pH 7.0-8.5) and there did not appear to be any toxic compounds present in the water. The pH levels in water samples obtained from the Cape Breton mines were found to be higher than expected, most samples being in the range 7.0-8.5. A few samples were acidic but the majority were closer to 8.0. The samples with low pH probably reflect the activities of sulphur oxidizing bacteria on the pyritic sulphur contained in the coal.

Methane oxidizing bacteria were found to be present in all water samples examined from the Western Canadian mines.

One type of organism was grown in large quantities and its growth requirements were partially characterized. The organism grew in a simple inorganic (Bushnell Haas) medium when incubated in a methane/air (20/80; v/v) mixture. The optimal pH (8.0) of this organism was very close to the average pH levels found in the Western Canadian mine waters.

The organisms were equally active when the distilled water used in the liquid medium was replaced with actual mine water. This showed that there were no inhibitory compounds present in the mine water.

The organisms retained 60% of their initial activity after a period of 100 days in contact with a number of low sulphur Western Canadian coal samples. However, they rapidly lost activity when in contact with an unwashed, high sulphur Cape Breton coal. This loss in activity is believed to be due to the oxidation of pyritic sulphur present in the coal, to sulphuric acid thus lowering the pH of the liquid phase to below that at which the methane oxidizing bacteria retain maximum activity.

When the methane oxidizing bacteria were added to partially degassed Western Canadian coals, the organisms oxidized the

methane as it desorbed from the coal. No methane was detectable after seven days whereas tests with no bacteria showed 7% methane concentration.

Experiments with a dynamic test set up showed that the organisms were able to reduce the levels of methane in air flowing through a coal bed by as much as 75% (2).

These results showed that biological methods were a viable alternative to conventional techniques. Thus further research was conducted with the following objectives:

(i) to carry out a detailed characterization of methane oxidizing microorganism cultures isolated from different coal mines in Western Canada. The study should emphasize the relationship between bacterial activity and changes in pH and temperature as observed in canadian coal mines. A pH range between 5.0 and 8.5 and a temperature range between 5 and 40°C should be covered. The study will include at least three mining operations of which two will be producing low volatile coals and one will be producing high volatile bituminous coal.

(ii) to isolate and culture methane oxidizing bacteria suitable for use with high sulphur coals. The study will include coals from both Prince and Lingan mines in Sydney, Nova Scotia.

(iii) to isolate and characterize the strains of methane oxidizing bacteria identified in the previous report and to establish the most suitable strain for use under a variety of mining conditions.

(iv) to carry out tests on a scaled up model simulating a coal storage silo. The model will hold 10 Kg of

coal during the test runs. The viablity of the biological method for removing methane will be evaluated based on the results of the scaled up tests.

EXPERIMENTAL

1 Coals studied

Mines from which water and coal samples were obtained are listed in Table 1. The types of coal mined at these sites are described in Table 2.

Water samples (500 ml) were collected in sterile containers which were tightly sealed and were kept as cool as possible until they were returned to the laboratory. Water was collected from both standing water and from water flowing down the walls of the mines.

Coal samples were collected from raw coal storage piles and from coal wash plants.

2 Growth of methane oxidizing bacteria

Two techniques were used to grow the methane oxidizing bacteria, namely, growth on solid and in liquid media.

Aliquots of mine water or single colony isolates were streaked onto Bushnell Haas agar plates. The solid medium was made up as follows:

Magnesium sulphate	0.2 g
Calcium chloride	0.02 g
Monopotassium phosphate	1.0 g
Dipotassium phosphate	1.0 g
Ammonium nitrate	1.0 g

Ferric chloride.....0.05 g
Bacto agar.....1.5 g
Tap water.....1000 mls

The plates were incubated in a vacuum desicator (under slightly reduced pressure to ensure proper sealing of the lid) in an atmosphere of methane and air (20:80; v/v). The plates were observed for growth after seven days.

Large quantites of cells were produced by adding an inoculum of rapidly growing cells into 1.2 l of liquid Bushnell Haas medium (same as solid except that no Bacto agar is added) in a New Brunswick Bioflow fermentor. Air was sparged through the system at a rate of 500 ml/minute. Stirring rate was 500 rpm and the temperature was controlled at 25°C. Methane was bled into the air lines at a rate sufficient to to give a concentration of 12-15% in the gas phase in the fermentor.

The fermentor was run in a fed-batch mode. After the cells had reached maximum density, 80% of the broth was removed and replaced with fresh sterile medium. This procedure was repeated twice weekly.

3 Adaption for growth at low pH

Aliquots of the fermentor culture were harvested by centrifugation and the pelleted cells were suspended in a small volume of Bushnell Haas medium. Five ml portions of this cell slurry were added to 100 mls of Bushnell Haas medium in 500 ml Erlenmeyer flasks fitted with side arms. The medium was adjusted to various pH levels between 4.0 and 7.5. The tops of the flasks were sealed with removable rubber membranes and the sidearms were fitted with serum stoppers to admit the entry and removal of gases. The

TABLE 1

Location of Mines from which Water and Coal Samples were Obtained

Company	Mine	Location
Westar Mining Ltd	Balmer North	SE British Columbia
	Balmer South	SE British columbia
Luscar Ltd	Cardinal River	W Central Alberta
	Coal Valley	W Central Alberta
Obed Mining Ltd	Obed Mountain	W Central Alberta
DEVCO	Lingan	Cape Breton, NS
	Prince	Cape Breton, NS

TABLE 2

Description of the Coals Used in the Study

Mine	Rank	Sulphur Content
Balmer North	Low volatile bituminous	0.31%
Balmer South	low volatile bituminous	0.31%
Cardinal River	low volatile bituminous	0.30%
Coal Valley	High volatile C bituminous	0.31%
Obed Mountain	High volatile bituminous	0.40%
Lingan	High volatile A bituminous	1.45%
Prince	High volatile A bituminous	5.1%
DEVCO Washplant	High volatile A bituminous	1.2%

atmosphere in the flasks was adjusted to 20% with methane and the flasks were incubated at 20°C. The atmosphere was replaced with a fresh methane air mixture twice a week. Every two weeks the cells were transferred to fresh medium, adjusted to the same pH as before.

After a few months the cultures obtained by growth at the low pH were grown in the fermentor as described above.

4 Characterization of the organisms

4.1 pH profile

Cells of the various strains tested were harvested by centrifugation and the pellets were suspended in a small volume of Bushnnell Haas medium. Aliquots of the cell suspension were added to Erlenmeyer flasks equipped with sidearms containing 100 ml of Bushnell Haas medium. The pH of the medium in the flasks was adjusted, before addition of the cells, to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. The flasks were sealed with removable rubber membranes and were fitted with serum caps. The atmosphere in the flasks was adjusted to 20% methane and the flasks were incubated over night at 20°C. After 18 hours of incubation the methane concentrations were measured by gas chromatography.

Three different strains were tested in this manner.

4.2 Temperature profile

The temperature profile of the organisms was tested in two different ways. Firstly, the organisms were added directly

to a sample of crushed Balmer North coal (200 g coal; 20 ml of a fermentor culture) in an Erlenmeyer flask with sidearm. The flask was fitted with a membrane and serum cap as described above. The atmosphere in the flask was adjusted to approximately 12% methane and the flask was incubated for 18 hours at the following temperatures: 5, 10 and 20°C. The residual methane concentrations were measured after 18 hours.

The second method involved testing organisms which were immobilized onto organic fibres (see immobilized cells below) and placed on the top of raw Prince coal. These cells were tested at a number of different temperatures between 5 and 40°C.

4.3 Microbiological characterization

Seven different strains of organisms were grown in liquid Bushnell Haas medium in small serum vials. The vials were sealed with serum caps to allow addition and withdrawl of gases. The atmosphere in the vials was adjusted to 20% methane and the vials were incubated at 20°C. After growth for a period of a week the bacterial cells were harvested and the following tests were conducted on the strains:

(i) growth at 45° and 37°C
(ii) colony morphology on agar plates
(iii) growth on methanol
(iv) detailed morphology under the electron microscope

5 Model silo experiments

Fifteen litre glass carboys were equipped with tight fitting

rubber stoppers and were used as model coal silos. Three copper tubes were inserted through the stopper and were set at different levels in the carboy, one going to the bottom, one was half way down and one was set just under the stopper. These tubes were used to add the methane to the silo and to take gas samples from the silo during a test. Thus gas samples could be taken from three levels, at the bottom of the coal pile, in the middle of the coal pile and from the airspace above the coal. A diagramatic representation of the silos is shown in Figure 1.

The silos were filled with approximately 10 Kg of partially degassed coal. Methane oxidizing bacteria were added to the coal (100 ml of fermentation broth per kilogram of coal) and methane was admitted to the silos to give a final concentration of 12%. The silos were kept at 20°C for 18 hours when gas samples were removed from the three sampling tubes for analyses of methane and oxygen concentrations. A control silo was run which contained only 100 ml of Bushnell Haas medium per kilogram of coal. The experiment was continued for a number of days to obtain information on the stability of the organisms.

The tests were repeated using a number of different coals from the following mines:

- Balmer North
- Cardinal River
- Obed Mountain
- DEVCO washplant.

6 Immobilized cells

In order to circumvent the problems associated with bacteria

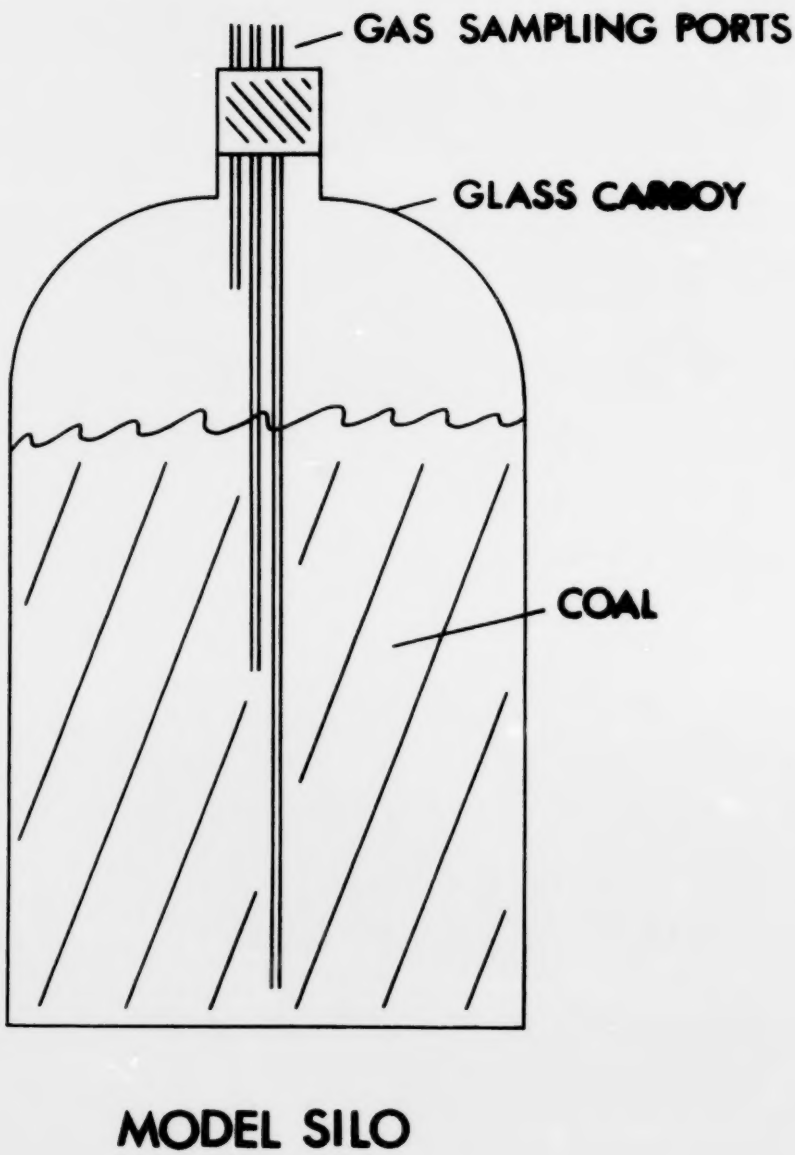


Figure 1. Diagramatic Representation of the Model Silo

in direct contact with high sulphur coals they were immobilized on a number of different supports. Four different suports were tried, a natural cellulose sponge, two synthetic foams, a synthetic fibrous filter material and fibre glass filter. The cells were immobilized by mixing aliquots of the cell culture with the material, incubating at 20°C for two days and decanting off the excess liquid. The immobilized cells were tested in flasks without coal and when placed in contact with Prince coal. The flasks were treated in the same manner as previously described for tests using small amounts of coal (2).

7 Economic assessment

The future commercial potential of this biological process depends on the cost to treat the coal. In order to arrive at an estimate for this cost it is necessary to calculate a number of variables for the process. Variables which will have to be quantitated will include the amount of bacterial cell culture which has to be added to the coal and the cost of producing this quantity of cells.

The first parameter was calculated by adding various amounts of cell culture to the coal and observing the effect of that quantity of cells. This was done by adding 1, 2, 3, 4 and 5 ml of a bacterial culture to 100 g of coal contained in small flasks. The flasks were filled with 12% methane and the amount oxidized in a period of 10 hours was determined. The value for 10 hours was then extrapolated so that a figure for 24 hours can be calculated. The data from these experiments could then be extrapolated to determine the quantity of bacterial culture required for a commercial scale operation.

The second parameter was caculated by setting up a fermentor culture of the organisms and withdrawing 10 ml aliquots daily and measuring the methane oxidizing activity of the aliquots as above. The optimum time for harvesting the organisms could then be determined.

These two parameters can then be combined to calculate costs for a commercial scale operation.

8 Analytical techniques

Necessary chemical and proximate analysis of the treated and untreated coals were carried out by a commercial laboratory following established ASTM methods.

Gases were analysed by gas chromatography using inhouse facilities. The experimental details of this technique and other methods are outlined in Appendix A.

RESULTS

1 Organisms

An important change in the character of the organisms was noted during their adaption to growing in the fermentor. When the organisms were grown in shake flasks or the small bioreactor used in the previous study the majority of the cells grew on the sides of the vessel. Once they were adapted to grow in the fermentor they grew in a suspended state and did not appear to be as "sticky" as the other cells and they could not be immobilized as successfully.

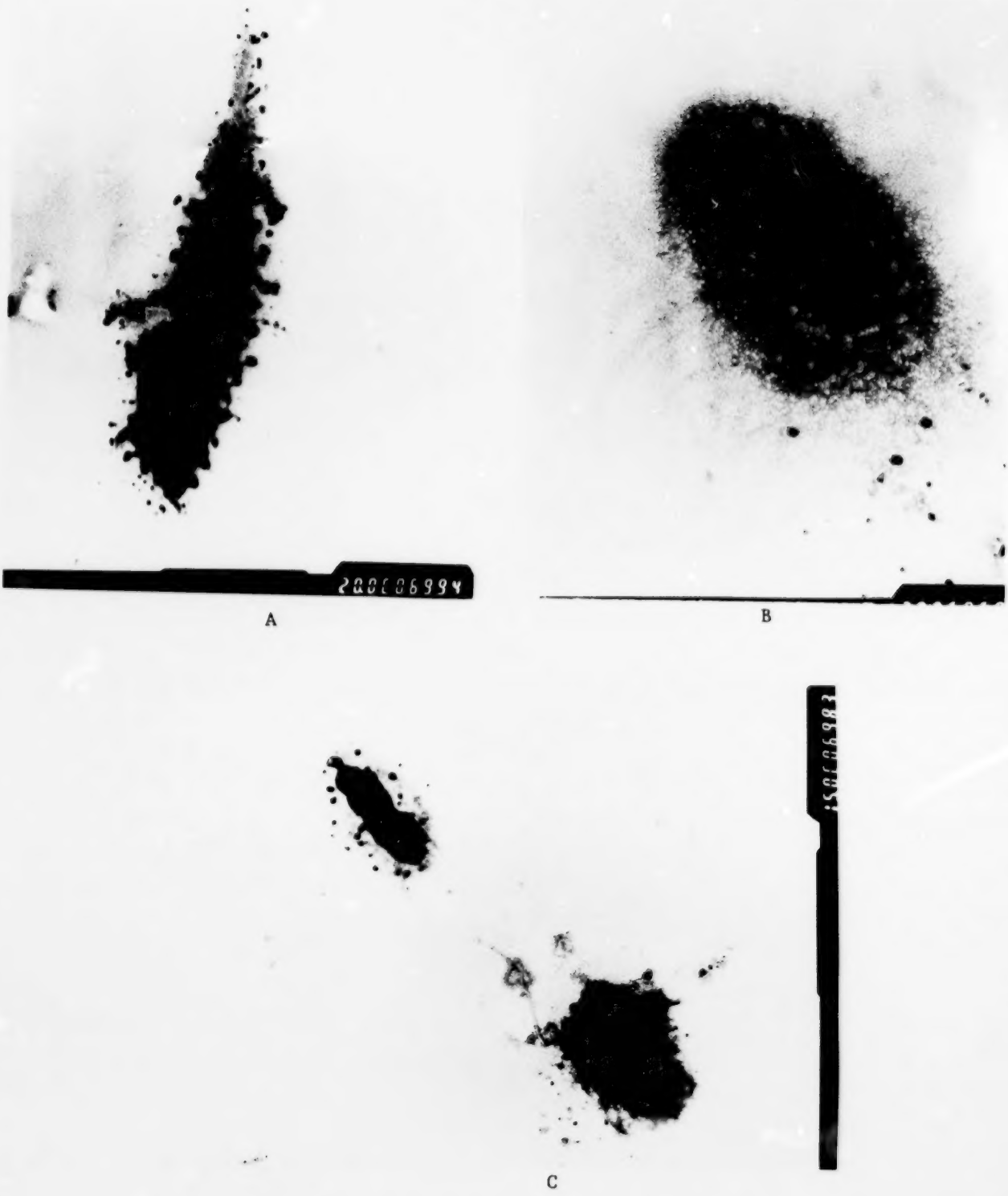
Seven other, morphologically distinct strains were isolated from Bushnell Haas agar plates and were further characterized. The fine structure of these strains as revealed by electron microscopy is shown in Plate 1.

2 Adaption for growth at low pH

Figure 2 shows that the organisms adapted for growth at low pH (4.5) had much more activity in the range 5.0-6.5 than the parent strain which was studied previously. All strains which had been cultured for four weeks at pH 4.5 were able to oxidize significant amounts of methane at pH 5.0.

3 Characterization of the organisms

The pH profiles of three strains are shown in Figure 2. The



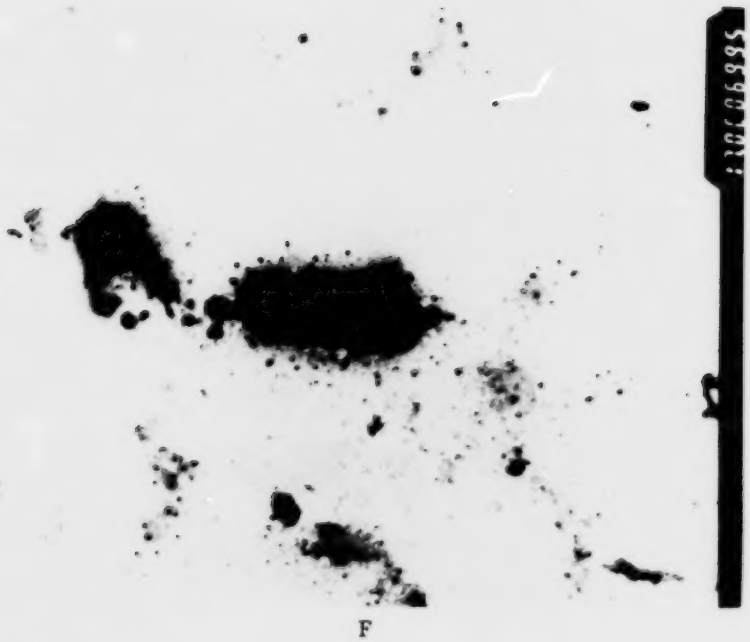
A B C

PLATE 1

Electron micrographs of various strains

A Yellow: B Reddish Pink: C Slimey

D E



F

PLATE 1 (Continued)

Electron micrographs of various strains

D Yellow: E Fermentor Culture: F White

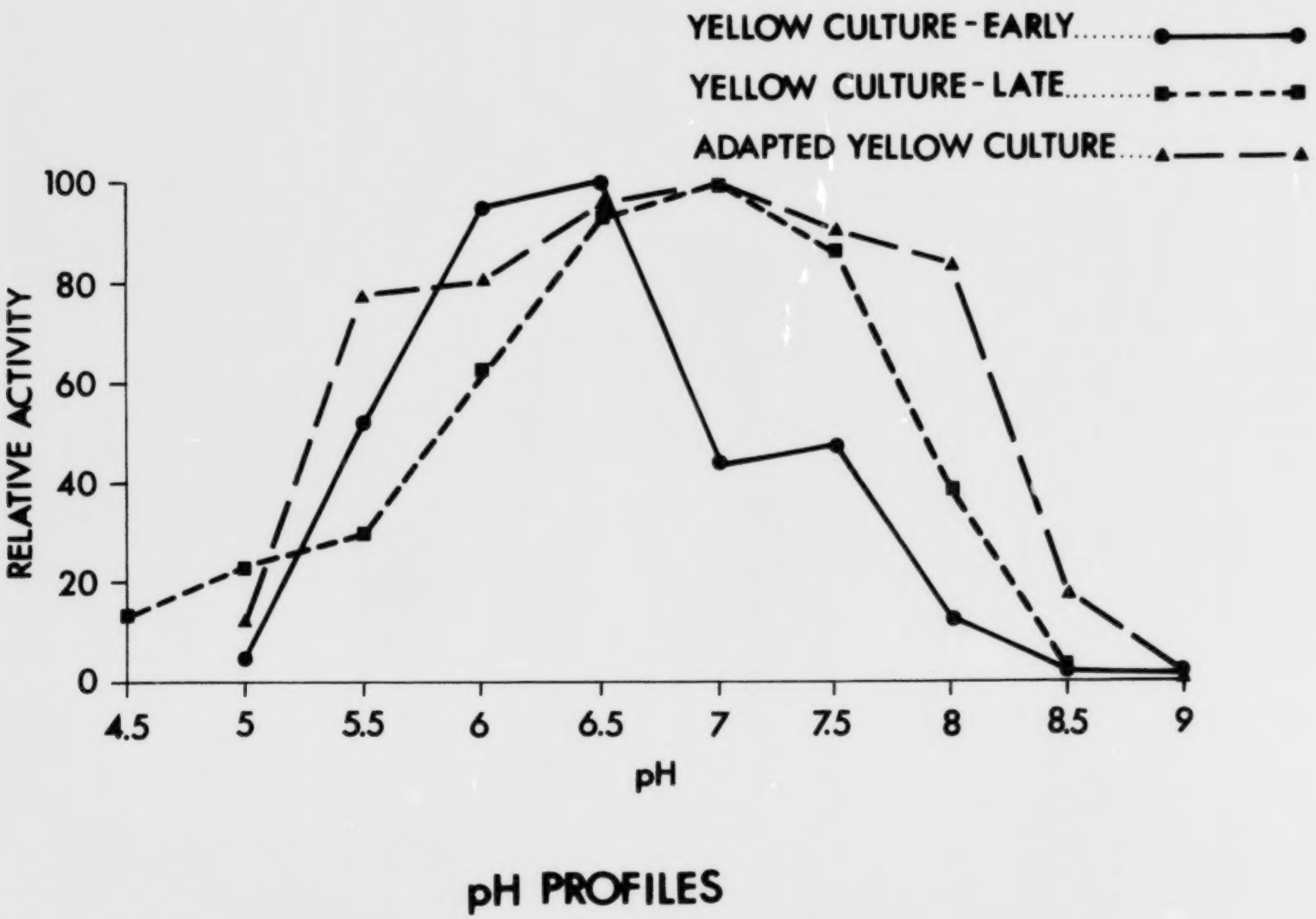


Figure 2. pH Profile of Various Strains of Organisms

pH profiles of these strains show good activity in the pH range 5.0-8.5. There was a very broad pH optimum between 6.0 and 8.0 where the activity remained greater than 80% of the maximum. All the strains tested showed much broader pH optima than the original strain which was isolated and tested in the previous study (2).

The temperature profile of the strain grown in the fermentor is shown in Figures 3 and 4. There did not appear to be any significant difference in temperature response whether the organisms were added directly to coal or were immobilized on a solid support. The temperature maximum for activity appeared to be approximately 33°C. The organisms were inactive at 40°C. However, this was only a temporary inactivation since once the temperature was reduced to 20°C they regained 100% of their original activity. The effects of freezing the organisms was also examined. After freezing at -20°C for 18 hours and subsequent thawing the organisms regained 100% of their initial activity.

The results of partial microbiological characterization are shown in Table 3. From this table it can be seen that all the strains are very closely related to one another.

Plate 1 shows detailed structure of the strains as revealed by electron-microscopic examination. Five of the strains showed appendages including pili and flagella. One strain had rather unusual appendages which could not be identified but resembled the prosecethae found in stalked bacteria.

4 Model silo experiments

The results of a number of experiments using the model silos are illustrated in Table 4. It can be seen from the table

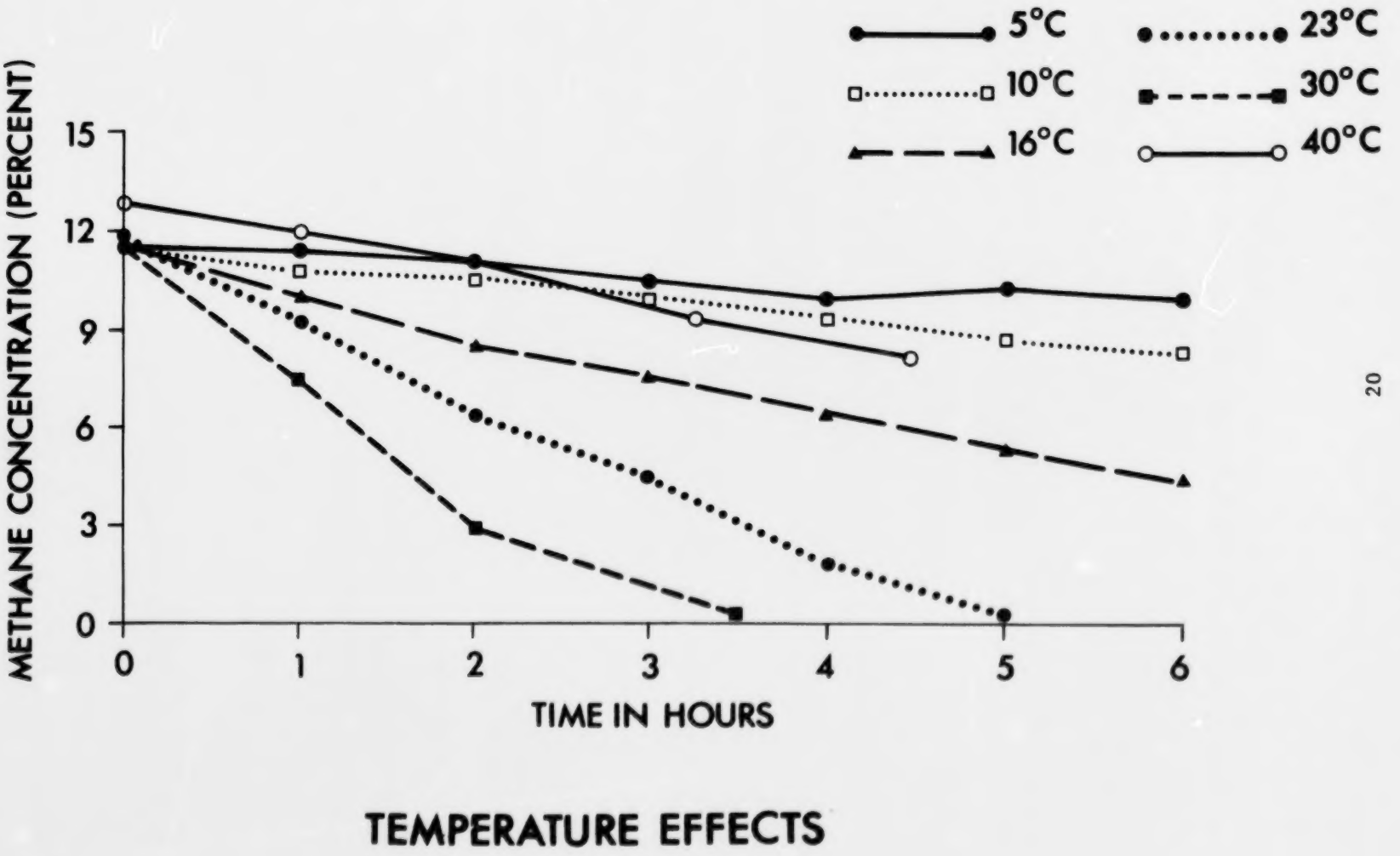
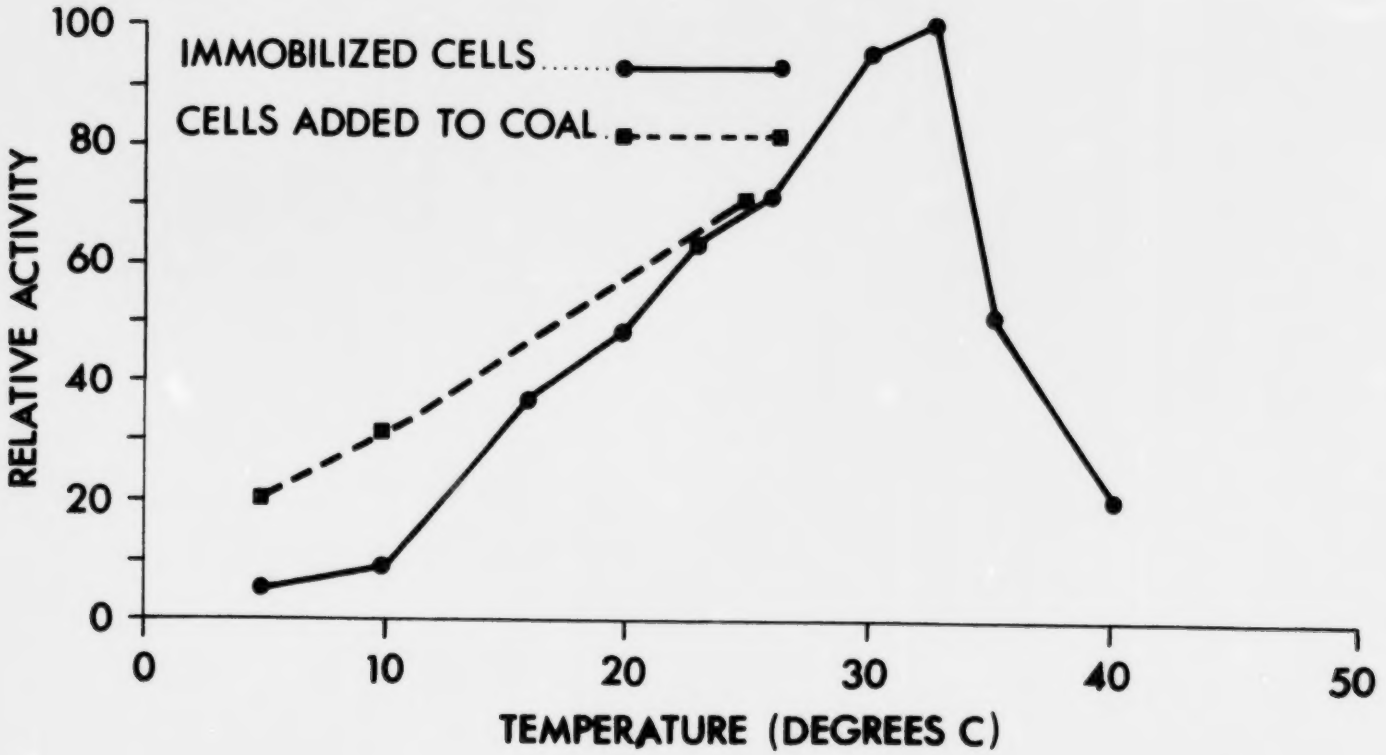


Figure 3. Effects of Temperature on the Microbial Activity



TEMPERATURE PROFILE

Figure 4. Temperature Profile of the Organisms

TABLE 3

MICROBIOLOGICAL CHARACTERIZATION

Strain	37°C	45°C	Flagella	Pili	Shape
Pale slimey	+	-	+	+	rods
Rough pink	++	-	+	+	"
Slimey	+++	-	+	+	"
Reddish pink	+++	-	+	+	"
Pink	+	-	+	+	"
White	+++	-	+	+	"
Yellow	+	-	+	+	"

TABLE 4

Summary of Model Silo Experiments

Coal	Initial Methane Concentration	Final Methane Concentration	Final Oxygen Concentration
Balmer North	11.27%	0.67%	3.28%
	13.04%	3.60%	0.00%
	12.33%	3.40%	1.80%
Coal Valley	9.20%	0.00%	3.28%
	12.72%	2.30%	0.28%
	13.94%	1.75%	2.50%
Obed Mountain	9.09%	0.00%	4.29%
	15.01%	6.84%	0.00%
	9.53%	0.00%	3.94%

that the organisms were equally active on all three of the Western Canadian coals tested. The organisms were as efficient at oxidizing methane in the large silos as they were in the small flasks used in the previous work (2). If the initial concentration of methane was 11% it could be routinely oxidized in a period of 18 hours at 20°C. Tests in which no bacteria were added lost approximately 1% methane.

The time course for methane oxidation in the model silo is illustrated in Figure 5.

The results could be fitted into one of two patterns. If the initial methane concentration was greater than 11%, there was residual methane in the silo but all or most of the oxygen was consumed. If the initial concentration of methane was less than 11%, it was all oxidized and 1-3% of oxygen remained. Thus oxygen limitation will determine the total amount of methane oxidized.

In the majority of the silo experiments, 10%, by weight, of fermentation broth was added to the coal. Under these conditions, 11% methane concentrations could be oxidized in an 18 hour period at 20°C.

When gas samples were withdrawn at the three different levels in the silo there was little difference in both the methane and the oxygen concentrations. Thus the rate of methane oxidation was not limited by the rate of diffusion of the methane or oxygen through the coal pile.

No large silo experiments were carried out on the raw Prince Mine coal. However, experiments in which 200 gram aliquots of DEVCO washplant coal (Lingan Mine) were used, showed that the organisms retained their activity for at least a period of 30 days. Typical data obtained from experiments using this coal are shown in Table 5. Thus the removal of the

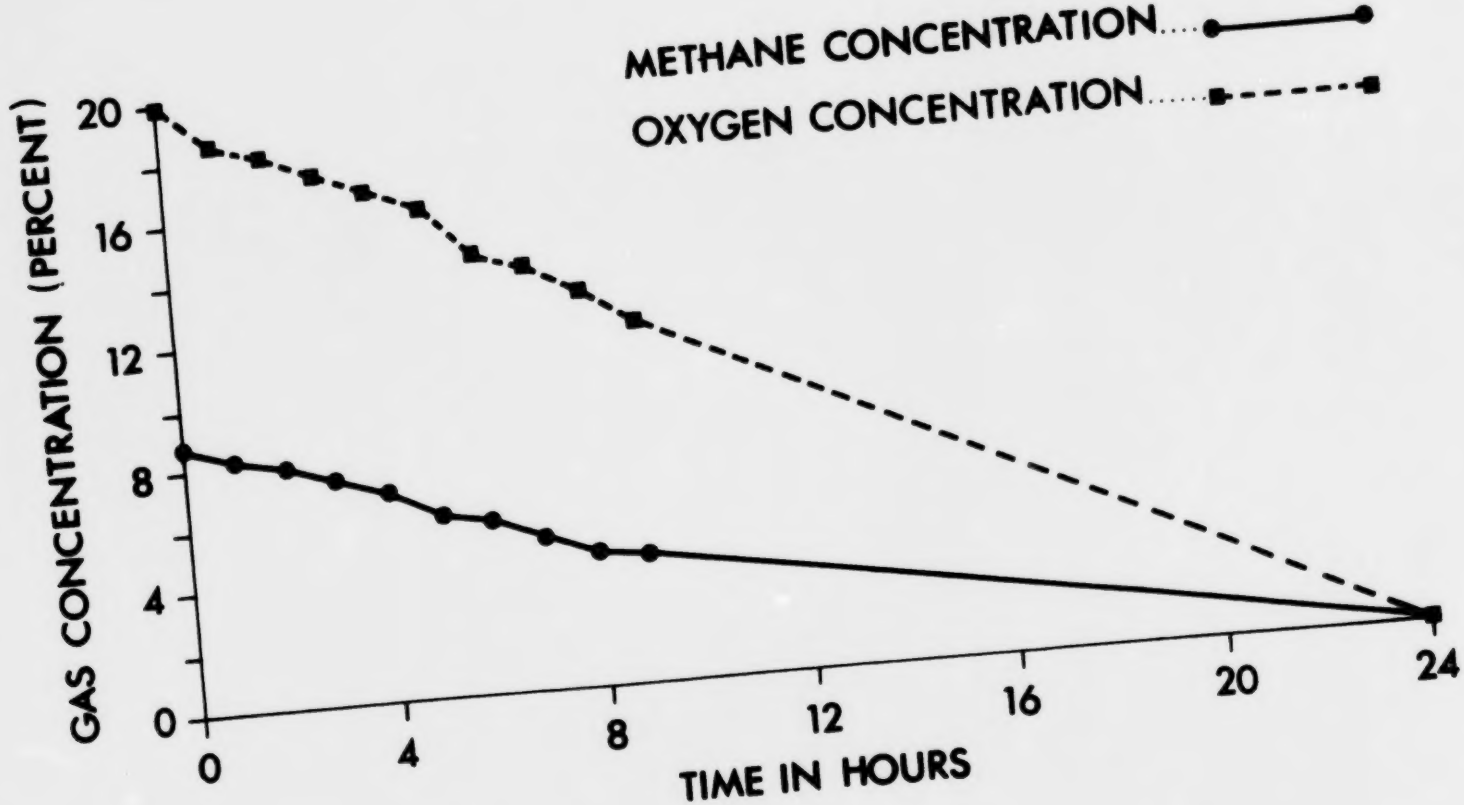


Figure 5. Rate of Methane Oxidation in the Model Silo

TABLE 5

Bacterial Activity in the Presence of DEVCO Washplant Coal

Test conditions	Methane* Concentration	Oxygen Concentration
Test with added bacteria	4.42%	0%
Test with no bacteria	12.04%	15.93%

* No added methane; methane desorbed from the coal

inorganic sulphur in the coal allowed the organisms to remain active when they were in contact with it.

5 Immobilized cells

A number of materials were tried for the immobilization of the organisms. A summary of the findings with a number of these materials is shown in Table 6. The results indicated that a synthetic fibrous filter material was found to be the best for use in the small flasks. Cells immobilized on this support and placed above raw coal obtained from Prince Mine retained 100% of their activity for a period of several months. When this material was tried on a larger scale (10 Kg silo) the results were disappointing. The material could not support the weight of the wet cells and compacted on the top of the coal greatly reducing the activity. It appears that the flow of methane and oxygen was restricted under these conditions.

6 Economic assessment

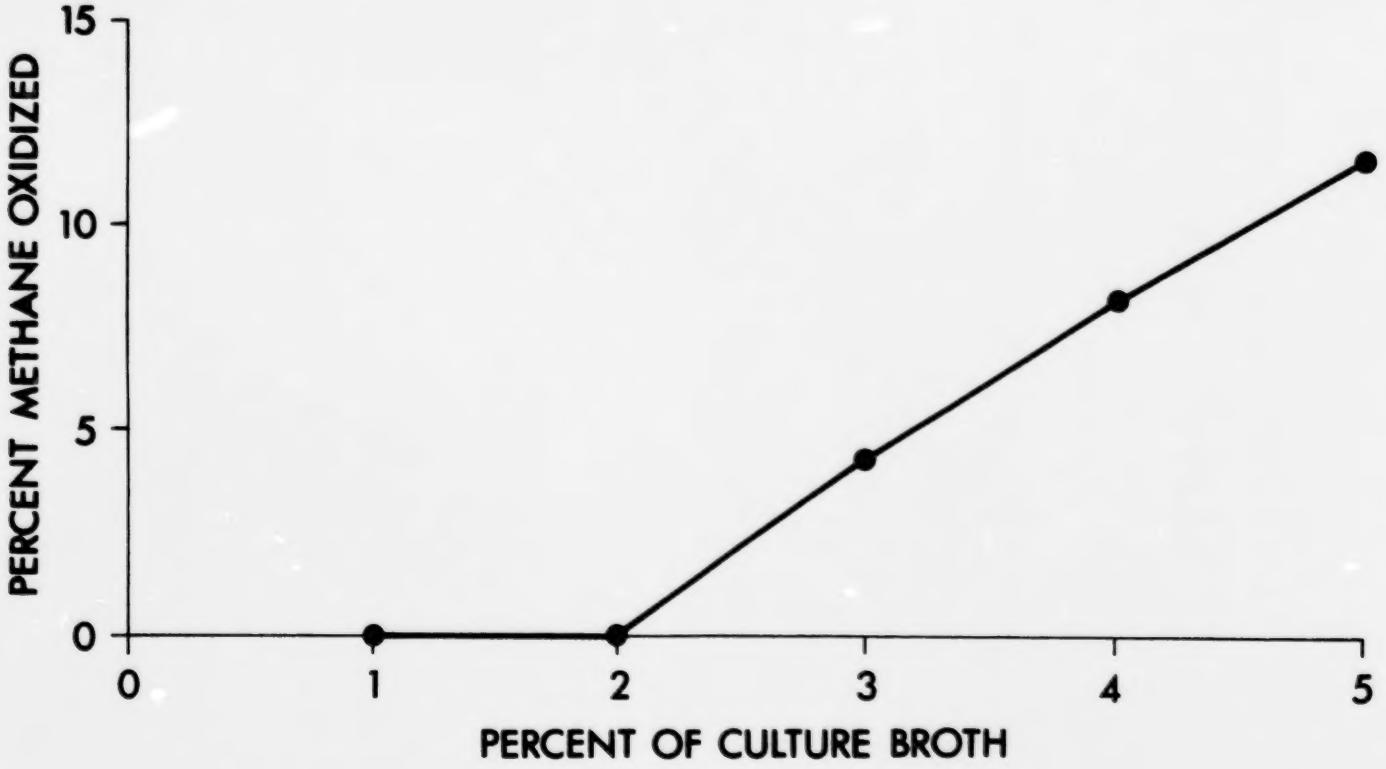
In order to derive an approximate cost for the biological methane treatment two parameters must be evaluated. Firstly, it is necessary to know the quantity of bacterial culture which must be added to a given quantity of coal and secondly, the cost of producing these bacteria must be calculated.

The effect of adding different amounts of culture broth to the coal is illustrated in Figure 6. It can be seen that a level of 3 mls of culture broth per 100 grams of coal gives effective oxidation rates. Figure 7 illustrates how the

TABLE 6

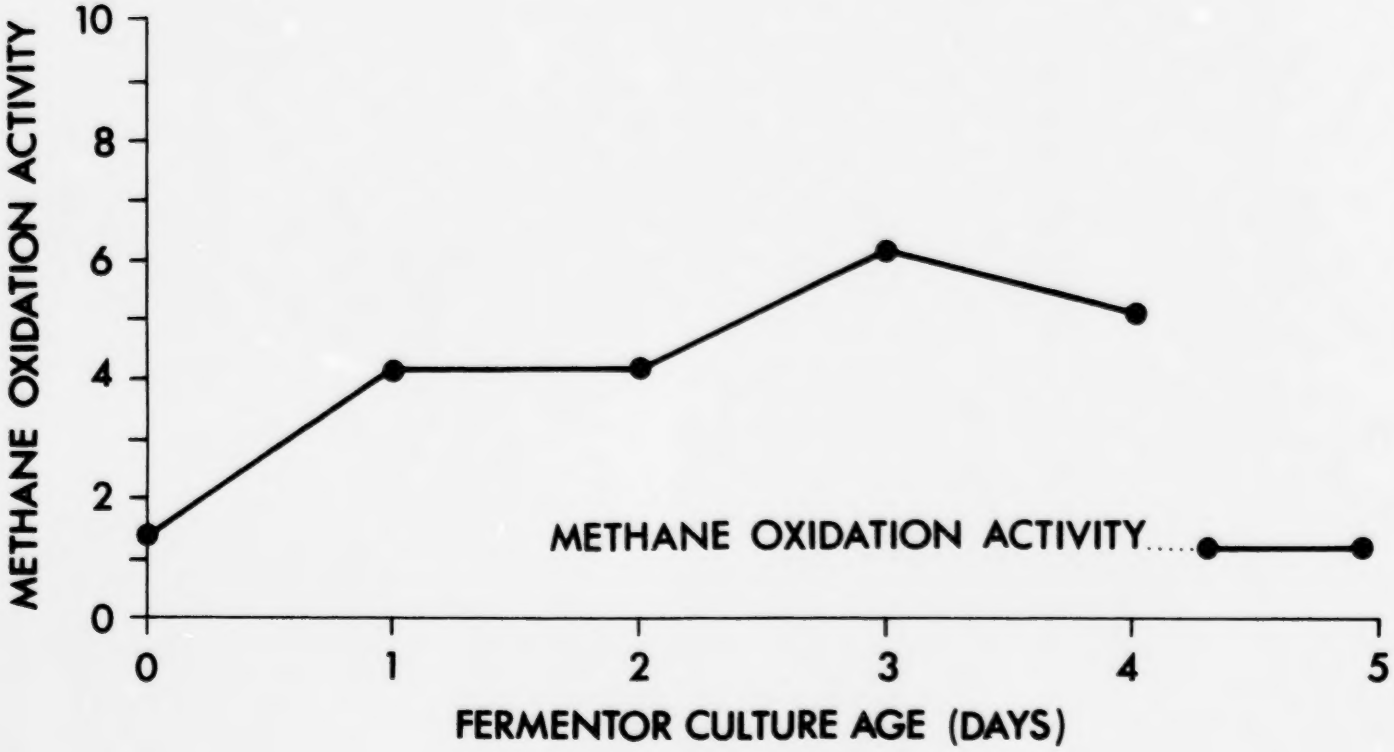
Results of Various Immobilization Supports

Supporting Material	Effects
Cellulose Sponge	Inactive
Synthetic foam A	Slightly active
Synthetic foam B	Slightly active
Synthetic fibres	Good activity



VARYING AMOUNTS OF CULTURE BROTH

Figure 6. The Effects of Adding Various Amounts of Bacterial Culture to Coal



FERMENTATION CHARACTERISTICS

Figure 7. Variation of the Bacterial Activity with Age of the Cells

methane oxidizing activity of the bacterial cells varies with culture age. Data from this experiment can provide insight as to the optimal time for harvesting the cells. A combination of the two sets of data i.e., a combination of the quantity of cells required and the time it takes to produce them in a fermentor, allows for the calculation of an approximate cost for a commercial scale operation. Using the data contained in Figures 6 and 7, an approximate cost of $0.50-1.00 per ton of coal was calculated.

Calculations on the costs to treat 1000 and 5000 tons per day of coal, are shown in Appendix B.

DISCUSSION

The results of the previous study (2) showed that strains of methane oxidizing bacteria isolated from coal mine waters could oxidize methane when in contact with low sulphur Western Canadian coals. The organisms retained their activity for a period in excess of 100 days when in contact with the coal. They were partially characterized and the conditions necessary to grow them established.

The study was continued so that more data could be obtained on the following parameters:

- o more detailed study on pH effects, particularily in the range 5.0-7.0
- o more data on temperature effects
- o scale up of the small silos to 10 Kg
- o develop alternative methods to treat high sulphur coal.

The results of this phase of the study were most positive and are discussed below.

The temperature profile of the organisms was an important parameter to study since in commercial scale operations it will not be practical to control the temperature, which will vary depending on the environmental conditions. The organisms studied in this project showed a temperature optimum in the range 30-33°C. Activity dropped off rapidly in the range 35-40°C and no activity was observed at 40°C. When the organisms were tested at lower temperatures there was a slow decline in activity, till, at 5°C, there was only about 10% of maximum activity. An important finding was that although the organisms were inactive outside the range 5-40°C, exposure to higher or lower temperatures did not

irreversibly inactivate the organisms since they regained their activity rapidly after exposure to temperatures of -20 and 40°C.

The effects of changing temperatures are important for two reasons. Firstly, cold weather may cool the coal to below the optimum and secondly, spontaneous heating may increase the temperature of the coal. The results described in this report show that the organisms can survive and be active in quite a broad range of temperatures. At present, no data could be obtained on the temperature variation inside coal silos or inside shipholds but, in general, it is believed to be above 5°C (3).

The pH profile of the organisms was studied in greater detail because the preliminary results reported in the previous study (2) suggested that they were only active in a rather narrow range (7.5-8.0). By culturing the organisms at a lower pH the pH optimum was broadened such that the organisms retained high activty in the range 6.0-8.0. The organisms were adapted to a lower pH to enable the organisms to be used in situations where the pH of the liquid phase was in the range 5.5-7.5.

When the small scale experiments were scaled up to 10 Kg of coal there was no detectable difference in the rate of methane oxidation between the small scale experiments and the larger silos. The organisms were equally active on all three Western Canadian coals.

The organisms were able to oxidize approximately 11% methane in 18 hours. When the gas composition was examined at different levels in the silo no differences were observed, indicating that, with the small silos at least, diffusion of methane and/or oxygen through the coal pile was not limiting the rate of oxidation. However, on a larger scale, the

availability of oxygen will determine how much methane will be oxidized since, once all the oxygen has been utilized, the excess methane desorbing from the coal will not be oxidized unless extra oxygen is available, either via leakage into the system or by ventilation.

In cases where the initial concentration of methane in the silo, or the total amount diffusing out of the coal, was greater than 11%, it was observed that there was always a residual amount of methane which could not be oxidized. When the oxygen levels were measured, it was found that in these cases, oxygen depletion was limiting the amount of methane oxidized. If the initial concentration of methane was less than 11%, all the methane was oxidized and 2-3% of oxygen remained. Thus the organisms are carrying out two useful functions. Firstly, where methane levels in excess of 11% are expected, the oxygen will be completely depleted thus lowering the risk of spontaneous combustion. Secondly, where the initial concentration of methane is less than 11% or where more oxygen will be available due to diffusion or leakage of air into the silo or shiphold, all the methane will be oxidized thus reducing the risk of methane explosions.

High levels of inorganic sulphur were shown to be inhibitory to the activity of the organisms (2). Two methods were tried to allow the organisms to remain active in the presence of this type of coal. As mentioned above, one of the ways was to adapt the organisms so that their activity was increased at lower pH values. A second way which was tested was to immobilize the bacterial cells onto a solid support which could be placed on top of the coal thus preventing direct contact between the organisms and the coal. This would ensure that the pH of the micro-environment was kept reasonably constant and would not decrease drastically.

A number of different supports, including a natural sponge, two synthetic foams and a synthetic fibre material were tried but only one proved successful. This was the synthetic fibre made into a filter material. The natural sponge completely inhibited the activity of the bacteria, possibly due to biocides incorporated into the material to prevent bacterial spoilage. The two foams only allowed the bacteria to retain 30% of their original activity.

Organisms grown in the small bio-reactor (not the fermentor--see later) could be easily immobilized onto this material. The organisms retained 100% of their activity when immobilized in this manner. The immobilized cells were placed on the top of high sulphur Prince coal where they were found to be completely active for extended periods. They retained 67% of their activity four months after being placed on top of the coal.

Organisms grown in the fermentor did not appear to be as easily immobilized since they lost their "stickiness" after continual subculture in the fermentor and preferred to grow in a suspended state rather than attached to solid surfaces.

The majority of the strains isolated appeared to be very closely related and may not be different species. The taxonomy of methane oxidizing bacteria is very complex and is presently being updated (4, 5). Thus no attempt will be made to classify the organisms other than to say that they are closely related to Methylomonas methanica. At a later date, samples of the strains could be sent to a number of researchers with expertise in this area who would identify the organisms according to new taxonomic principles. Such detailed characterization is beyond the scope of this project at present.

The previous report (2) indicated that biological control of

methane did not appear to work when the organisms were added to coals containing high levels of sulphur. Thus ways were sought to allow the process to be adapted to treat this type of coal. As described above, two methods were tried, adapting the organisms to remain active at lower pH and immobilizing them so that they did not come in direct contact with the coal. The above methods used unwashed, raw coal. A third way to adapt the method will be to use it with coals which have had the inorganic sulphur removed. Thus when coal from the Cape Breton wash plant was tested it was found that the organisms were active in the presence of this coal and that the activity was stable for a period of at least one month.

The three methods to treat high sulphur coal are summarized below:

- adapt the organisms to be more active at lower pH
- immobilize the cells so that they are not in direct contact with the coal
- remove the inorganic sulphur from the coal.

A preliminary economic assessment of the biological method has been made and has shown that the cost for treating one ton of coal will be in the range $0.50-1.00. This cost will vary with a number of factors such as:

- level of methane in the coal to be treated
- total amount of coal to be treated per day
- rate that is acceptable for methane oxidation

Coals with high levels of methane, (or which have high methane desorption rates) will require greater amounts of the bacterial cells than coals with less methane since the rate of methane oxidation is proportional to the number of

bacterial cells added. If the rate of methane oxidation is not critical and lower rates can be tolerated (e.g. in stockpiles or silos) then less bacteria can be used.

The total amount of coal to be treated will also affect the cost. Larger quantities of coal will require larger fermentation facilities which will result in significant reductions in unit costs. Capital costs account for approximately 50% of the total cost of treating the coal, thus the cost will decrease substantially because of economies achieved in larger scale plants. The most cost effective utilization will be if the fermentation facilities are operated on a full time basis.

The previous report (2) had shown that the organisms were not active when placed in contact with high sulphur Cape Breton coals. Results described above show that the organisms are active when applied to coal obtained from the DEVCO washplant where most of the inorganic sulphur has been removed and the sulphur content has been reduced to less than 1.2% (6). This is the coal which is loaded into the coal carrying ships and which is presently the cause of concern by both DEVCO and Transport Canada. Thus it would appear that there should be no problems in treating this coal on a larger scale.

CONCLUSIONS

1 Small scale experiments were sucessfully scaled up to allow treatment of 10 Kg quantities of coal. The rates of methane oxidation in the larger models were as high as those observed in the small tests. No differences were noted for gas samples withdrawn either in the airspace above the coal or from within the coal pile.

2 The pH activity profile of the strain of bacteria used in most of this study was broadened from that used in the previous work. By culturing the organisms at lower pH, significant activity was found at lower values, especially in the range 5.0-6.5. The organisms were most active in the range 6.0-8.0.

3 The organisms were more fully characterized, particularily their temperature and pH profiles. Optimum temperature for bacterial activity was shown to be at 33°C. Activity dropped rapidly when the temperature was raised above the optimum but only dropped slowly when it was lowered. The organisms were inactive at extremes of temperature, e.g. -20 and 40°C but recovered when the temperature was adjusted to more favourable values.

4 Methods were adopted to allow the organisms to be utilized with high sulphur Cape Breton coal. Specifically, three methods were found to allow the organisms to be used with the Cape Breton coals. Firstly, the pH profile of the organisms was adapted to lower pH values. Secondly, methods were found to immobilize the organisms so that they did not come in contact with the coal. Thirdly, if the inorganic sulphur was removed the resulting coal did not inactivate the organisms.

RECOMMENDATIONS FOR FURTHER RESEARCH

Based on the results described in this report, the following recommendations are made for further research.

1 Further scale up of the model silo is justified. It is recommended that the silos be scaled up to treat 2-3 tons of coal. Two Western Canadian coals (Balmer North and Coal Valley coals) and one from Cape Breton (DEVCO washplant) should be tested at this scale.

2 A simple air lift type of bioreactor with a 50 gallon capacity should be designed and built. This bioreactor should be large enough to produce sufficient cells to treat 2-3 tons of coal.

3 Experiments should be conducted with both the 1 litre fermentor and the 50 gallon bioreactor to determine optimal conditions for producing the organisms in large quantities.

4 More experimental data are required on the changes noticed when the organisms were grown in the fermentor. In particular, more data should be collected on the differences observed in the methane oxidation rates between the "sticky" cells and the freely suspended cells.

5 A more accurate economic assessment should be made and detailed plans for a plant to treat 1000 tons per day of coal should be drawn up.

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APPENDIX A

Analytical methods

Gases were analyzed on a Perkin Elmer Sigma 3B gas chromatograph using the following conditions:

column	Supelco carbosieve SII 10 x 1/8' s.s.
oven temperature	65°C for oxygen, 225°C for methane and carbon dioxide
injector temperature	250°C
detector temperature	250°C
carrier gas	helium at 30 ml/minute
detector type	hot wire
sample loop	0.1 ml

pH measurement was made using a combination electrode and a Fisher Accumet pH meter (model 425 Digital).

APPENDIX B

Economic Assessment

The costs to treat a ton of coal were calculated as follows. Assume 5000 tons of coal to be treated daily, 3% by weight of culture broth added to the coal.

Volume of broth required per day is 33,600 gallons.

If broth takes three days to grow, then daily volume is 100000 gallons.

A plant to produce this volume of material can be bulit for $5000000* excluding a bulding). This can be amortized over 10 years at a cost of $500000 per year.

Costs to run the plant for a year:

Depreciation	$500000
Labour	150000
Raw materials	200000
Operating costs	50000
TOTAL COSTS	$900000

Thus it will cost approximately $900000 to treat 1000000 to 1750000 tons of coal (depending on through put). This can be equated to a per ton cost of $0.51-0.90.

* Costs estimated from pilot scale fermentor constructed by Gemini Biochemical Research Ltd.

Assume 1000 tons of coal to be treated daily, 3% by weight of culture broth added to the coal.

Volume of broth required per day is 6720 gallons.

If broth takes three days to grow, then daily volume is 20000 gallons.

A plant to produce this volume of material can be built for $1000000* (excluding a building). This can be amortized over 10 years at a cost of $100000 per year.

Costs to run the plant for a year:

Depreciation	$100000
Labour	125000
Raw materials	60000
Operating costs	30000
TOTAL COSTS	$315000

Thus it will cost approximately $315000 to treat 200000 to 350000 tons of coal (depending on through put). This can be equated to a per ton cost of $0.80-1.40.

* Costs estimated from pilot scale fermentor constructed by Gemini Biochemical Research Ltd.